($\lambda$ = 365 nm), the selective disassembly of **1** occurs while the fibrillar structure of **2** remains intact (Fig. 1b(iv)). The gel phase is preserved, although it loses the strength of the multicomponent system. It is interesting to note that the viscoelastic properties and the morphology of the modified gel — the multicomponent gel {**1**+**2**} from which **1** has been selectively removed (Fig. 1b(iv)) — fully match those of the gel formed from **2** alone. This implies the complete spatial resolution of the two types of fibre, making each of them numb to missing neighbours.

In a particularly enticing follow-up experiment, the researchers showed that it was also possible to pattern the blend by preventing disassembly in a covered surface by using a photo-mask (Fig. 1b(v)). A variety of photoswitchable gelators had previously been devised, and the selective irradiation of their gels through a mask had been used to disassemble them at specific locations. But in a single-component system this meant a gel–sol transition for the material at the macroscopic level. In contrast, here the second network of fibres (assembled from **2**), which remains intact, holds the macroscopic material together, resulting in a patterned gel instead.

Thus, chemically triggered sequential molecular assembly of the components and conservation of the self-identity in macroscopic scale has now been firmly established. This is certainly attractive for supramolecular chemists as it emphatically advertises the strength of molecular programming approaches. The type of coexistence of multiple functionalities in a single material without macroscopic phase separation illustrated in Fig. 1b(iii) is promising, for example in plastic solar cells, where one can imagine the two different functionalities being n- and p-type semiconductors. Such a material should facilitate photoinduced charge-separation in the junctions of the two types of fibre, and concurrently minimize the probability of recombination as the positive and negative charge-carriers would find uninterrupted pathways to reach the respective electrodes. Furthermore, the fact that the two different networks are separately addressable (Fig. 1b(iv)) evokes the possibility, albeit in its infancy, of exploring supramolecular imprinting. One can envisage that this will present a wider scope than that of molecular imprinting[9], through the ability to extend the stamping over much longer length scales. It will perhaps also even be possible to tune the dimension and character of materials by structural fine-tuning of the building block forming the sacrificial fibres.

Such exciting and varied possibilities are set to find use in emerging applications of supramolecular materials[10], such as in miniaturized organic electronics, biotechnology, tissue engineering and catalysis. ❐

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BIOANALYTICAL CHEMISTRY

# Eavesdropping on interactions

A method for directly probing binding interactions in free solution, without the need for chemical tagging, offers exciting opportunities for non-perturbative analyses of biomolecules in their native state.

Enrique Valera and Ryan C. Bailey

Technologies that are able to monitor interactions between biomolecules and binding partners, be those small molecules, native ligands or engineered receptors such as antibodies, have wide-ranging applications throughout chemical biology, biophysics and clinical diagnostics. Owing to this importance, molecular characterization techniques have become widespread in the commercial marketplace; however, tremendous opportunities still exist to develop novel approaches that avoid some of the limitations of existing methods — particularly in the area of creating cost-effective and experimentally simple approaches to characterizing biomolecular interactions.

A common approach to monitoring biomolecular binding interactions involves the use of tags or labels covalently linked to one of the binding partners, which enables either monitoring by fluorescence or isolation via affinity interactions. Fluorescent labels enable the ultimate in sensitivity, and single-molecule fluorescent methods have become mainstream over the past decade[1]. Furthermore, fluorescence imaging allows for the spatial identification of specific biomolecular interactions. Despite this wide utility, the covalent modification of biological molecules can significantly perturb native binding interactions. For example, a recent study found that the kinetics and spatial distribution of protein–protein binding interactions was significantly altered by the incorporation of a fluorescent dye[2].

With the aim of probing such biomolecular interactions more innocuously, there has been a flurry of recent activity towards the development and deployment of label-free technologies for binding analysis. These technologies can be broadly defined as those that do not require the use of a dye or affinity tag to either of the binding partners. Leading the way in the commercial domain is surface plasmon resonance (SPR)[3]. In SPR, one of the binding partners is covalently bound to a gold film, and interactions at the interface are measured on account of the local change in refractive index upon complex formation. There are also a number of emerging optical and electrochemical methods that similarly monitor the interactions of biomolecules at interfaces, such as waveguides and electrodes, respectively. This actively expanding area has already led to many exciting new technology developments; however, a shared concern with all of these approaches is the need to physically attach one of the binding partners to a surface,

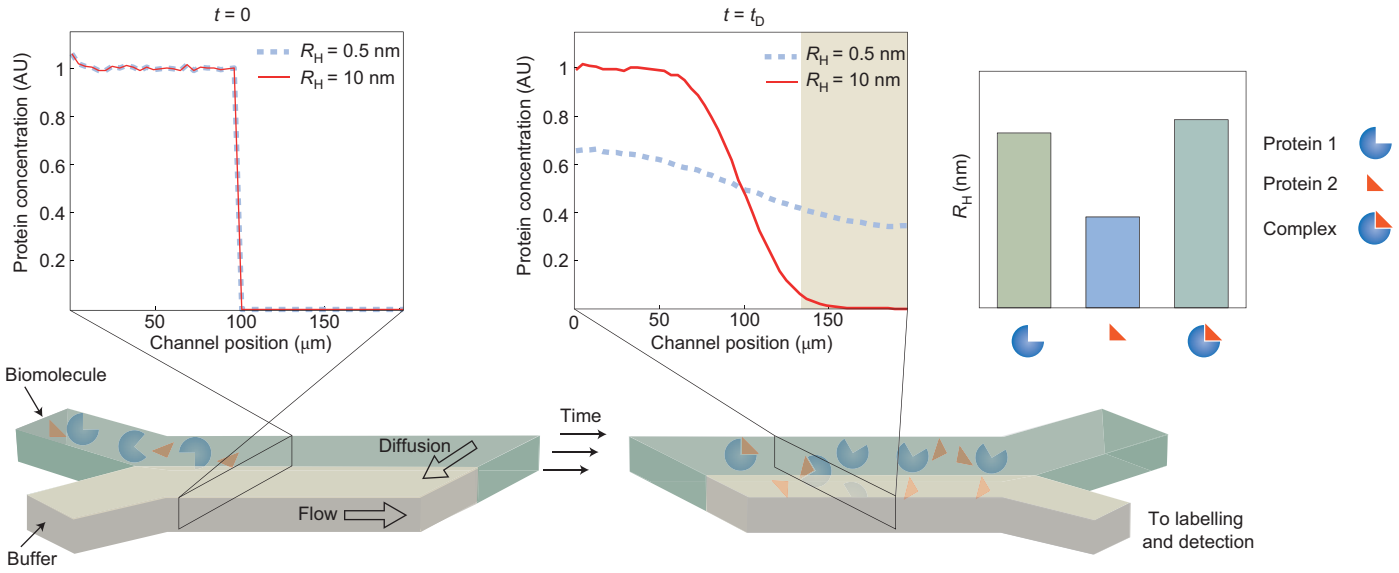


**Figure 1 |** Flow-based sizing allows label-free analysis of biomolecular interactions. A solution of the biomolecules being studied is merged with a second stream of buffer. Diffusion perpendicular to the flow direction leads to separation based on hydrodynamic radius ($R_H$). The graphs show the distribution of molecules at time $t = 0$ and at a later time $t_D$ (dictated by channel length and flow rate). After allowing time for this diffusion, a fraction of the flow can be separated for analysis by quantitative fluorescent labelling. Comparison of the (known) input concentrations of biomolecules to the fractions of each protein, and the complex in the output can reveal the strength of the binding between them.

which once again risks perturbing the native biomolecular interactions.

By contrast, a number of surface-less, label-free methods exist, with major players including mass spectrometry[4], isothermal titration calorimetry[5], and native fluorescence capillary electrophoresis[6]. Each of these techniques is, however, accompanied by at least one significant detraction, including cost and being in the gas phase, large sample requirements, and the necessity for sufficient intrinsic fluorescence, respectively. Robust, cost-effective and simple-to-use methods for interrogating biomolecular interactions in free solution without perturbing native binding conditions therefore represent a grand challenge for biomolecular interaction monitoring.

Writing in *Nature Chemistry*, Dobson, Knowles and colleagues describe[7] a clever approach for latent monitoring of biomolecular interactions with very high sensitivity using a relatively simple microfluidic device. The fundamental advance therein is the utilization of diffusional sizing to spatially separate formed biomolecular complexes from unbound partners, after which quantitative labelling with a fluorescent tag allows for attomole detection using standard fluorescent microscopy. Importantly, the diffusional sizing is performed in the absence of any label and therefore the native biomolecular interactions are being directly accessed under physiologically relevant and non-perturbative conditions, before being quantified using fluorescence.

The device itself is refreshingly simple (Fig. 1). A microfluidic channel containing the biomolecules of interest is mixed with another channel containing only buffer under conditions in which there is no convective mixing. As the solutions flow side-by-side, biomolecules are transported perpendicular to the direction of the flow channel exclusively by diffusion. After a time period rigorously defined by the length of the microfluidic channel, a small amount of the total fluid flow is diverted for detection, which is achieved using quantitative fluorescent tagging.

For this approach to work, each protein must be labelled with a uniform number of fluorophores. Thus, the choice of tagging chemistry is important, and the use of a reaction between primary amines and an *ortho*-phthalaldehyde dye is a wise choice. The diverted flow from the main channel is rapidly mixed with a denaturant, allowing access to all the free amines, and reacted with the dye before simple fluorescent quantification. Since the amount of biomolecule at any particular region within the channel is determined by diffusion alone, and this is related to the fundamental molecular property of hydrodynamic radius, the approach can effectively size biomolecules and biomolecular complexes in free solution. It is worth noting that the diverted fraction of the flow could be analysed using a number of alternative techniques, including mass spectrometry. However, the fluorescence labelling approach described so far has advantages in terms of cost, rapidity and simplicity.

Knowles and co-workers rigorously demonstrate the ability to accurately size a number of proteins; however, the ultimate potential of this approach becomes further apparent as they apply their technology to the latent analysis of biomolecular interactions. As test cases, they applied this approach to monitoring the dimerization of bovine insulin hormone as well as the previously undescribed interaction between α-synuclein and a single-domain antibody, termed a nanobody. The particular nanobody used was previously developed as a probe for subtle conformational differences accompanying α-synuclein fibril maturation, which have important consequences in severe neurological diseases, including both Parkinson's and Alzheimer's. Using their flow-based sizing approach, the research team were able to both confirm binding and determine the hydrodynamic radius of the protein–protein complex.

Although they have not used this technology to determine key kinetic or thermodynamic properties of the complex — as is often performed using many of the alternative label-free methods described above — it is easy to imagine ways of deploying the technology to extract such parameters. A minor detraction of the technology is its requirement for the presence

of amine groups for labelling; however, the constant presence of N-terminal amines as well as the near ubiquity of lysines within proteins greatly obviates this concern. That said, alternative labelling chemistries would need to be deployed to further extend this technique to other classes of biomolecule, such as carbohydrates.

We should also mention an alternative and equally exciting approach for the label-free, free-solution characterization of biomolecular interactions: back-scattering interferometry (BSI)[8]. This is an inteferometric technique that uses only an inexpensive laser, CCD camera and a simple microfluidic device. It does not require any labelling whatsoever and has been previously deployed to interrogate a wide and diverse range of biomolecular interactions. However, it also must be mentioned that several recent manuscripts have raised questions about the mechanism of BSI[9], having implications on its utility in some applications.

Techniques that can probe biomolecular interactions in a non-perturbative, native environment will undoubtedly find increasing adoption in many applications throughout drug discovery, biophysics and clinical diagnostics. It is an exciting time to eavesdrop on native biomolecular interactions, and new methods continue to emerge as powerful weapons in the technological arsenal. ❐

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BIOMINERALIZATION

# Nanocrystals by design

Nanocrystals with precisely defined structures offer promise as components of advanced materials yet they are challenging to create. Now, a nanocrystal made up of seven cadmium and twelve chloride ions has been synthesized via a biotemplating approach that uses a *de novo* designed protein.

Li Shang and Gerd Ulrich Nienhaus

The assembly of complex structures from simpler building blocks — known as the bottom-up approach — is a promising strategy to fabricate nanomaterials for use in many different fields, including electronics, photonics and energy conversion and storage. Nanocrystals play a key role in these efforts because they often possess physicochemical properties relevant to their function that are size- and shape-dependent, and markedly different from their bulk counterparts. However, as nanocrystals grow, so does the probability that defects will be incorporated thereby giving rise to heterogeneity. In contrast, industrial applications usually require a reliable and robust synthetic route, so that nanocrystals with well-defined physical dimensions and surface chemistries can be cheaply synthesized in bulk quantities. To this end, materials chemists have made enormous efforts to develop ways to produce a wide array of nanocrystals in a tightly controlled fashion[1,2]. Nevertheless, despite these endeavours the products are typically heterogeneous in size, shape and surface structure[3] and, notwithstanding a few notable examples[4,5], we are still far from having atom-level control over nanocrystal growth.

Now, writing in *Angewandte Chemie*, a team led by Arnout Voet, Kam Zhang and Jeremy Tame report the formation of a precise 19-atom nanocrystal within an artificially designed protein assembly via a biomineralization strategy[6]. Key to their approach is the artificial β-propeller protein Pizza, which the group previously created by computational design[7]. The original version, Pizza6, has six-fold rotational symmetry because it consists of six tandem copies of a 42-residue domain, the 'blades' of the propeller, containing four β-sheets each. The modified polypeptide used here, nvPizza2-S16S58, includes only two nearly identical domains per chain, so that a trimeric assembly of this variant reconstitutes the overall Pizza6 structure (Fig. 1a). A single mutation was introduced by replacing serine 58 (S58) with histidine in the polypeptide chain to enhance the tendency to form such trimers. The rationale behind this modification is that it creates an octahedral metal ion binding site on the symmetry axis formed by the three H58 residues of the trimer and three water molecules.

High-resolution X-ray crystallography did indeed reveal the formation of trimers when the modified protein was crystallized from a solution containing cadmium ions (0.2 M). In addition, the trimers were observed to dimerize in a sandwich structure. Surprisingly, a nanocrystal was found in the dimer interface, composed of seven cadmium ions (in a plane perpendicular to the six-fold axis) and twelve chloride ions (Fig. 1b). At its periphery, the nanocrystal was coordinated by a set of twelve symmetrically arranged histidines (H31, H73). The intriguing protein–nanocrystal structure vividly shows that polypeptide-based biomineralization can be a powerful approach to synthesize nanocrystals with highly defined structural properties.

In natural biosystems, inorganic nanostructures are typically created by polypeptides serving as binding templates and nucleation sites for crystal-forming ions, providing only limited space so as to inhibit further growth of the nanocluster core. A popular example is ferritin, a protein which stores iron inside cells[8]. Its 24 polypeptide chains are arranged as a hollow shell enclosing a spherical interior cavity with a diameter of 8 nm (Fig. 1c), in which a few thousand iron ions can be stored in the form of nanocrystals. This remarkable protein has been harnessed as a nanoreactor for the synthesis of inorganic nanoparticles with highly defined dimensions (Fig. 1d). Mann and co-workers employed ferritin to synthesize iron sulfide particles as well as manganese and uranium oxohydroxide nanocrystals[9], and following this pioneering work a